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### ②④ METHOD FOR DIAGNOSIS OF HEPATIC CANCER OR HEPATOCIRRHOSIS.

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## Description

The invention relates to a method for diagnosing cancerous diseases of the liver based on the increase in the amount of UDP-N-acetylglucosamine : glycoprotein N-acetylglucosaminyltransferase (hereinafter abbreviated as Gn-T-III) in serum.

The inventive method allows simple diagnosis of hepatic cancer (hepatocirrhosis) by measuring the increase in the amount of Gn-T-III in serum, and hence will be of much benefit to the medical and diagnostic fields.

GOT, GPT, LDH, ChE and many other test items have been adopted for general diagnosis of hepatic functions.

These test items, however, are no more than to check the comparative degree of hepatic functions, and are far from direct diagnosis of hepatic diseases, particularly hepatic cancer.

Measurement of tumor markers, such as AFP and CEA, is also known to be useful for the diagnosis of hepatic cancer and has been put into practice.

But these conventional tumor markers show a positivity rate of 60% at the highest, making early diagnosis almost impossible.

The above abbreviations have the following meanings:

GOT: glutamic-oxaloacetic transaminase;

GPT: glutamic-pyruvic transaminase;

LDH: lactate dehydrogenase;

ChE: cholesterol ester;

AFP: a fetoprotein;

CEA: carcinoembryonic antigen.

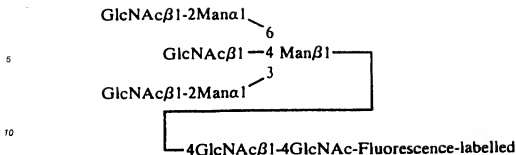
In "Cancer Research", vol. 39, June 1979, pgs 1943-1951 it is reported about glycosyltransferase and glycosidase activities in ovarian cancer patients in order to elucidate the mechanism of appearance of abnormal glycoproteins in cancer; thus activities of glycoprotein glycosyltransferases and glycosidases were determined in the homogenates prepared from normal ovaries and ovarian epithelial adenocarcinoma. Significantly high activities of these enzymes have been observed in some cases as well as substantially elevated activities above normal controls among the glycosidases; it is reported that the level of N-acetylglucosaminyl transferases is elevated in the sera of 80 to 90 % of the patients known to suffer from ovarian cancer. Generally, the activity of GlcNAc transferase was measured by the use of two substrates. The substrates have not homogenous structures. The structures of the substrates are undetermined; the same refers to the structure of a compound produced by this reaction. Further GlcNAc transferase is a generic name including plural different enzymes since plural kinds of GlcNAc transferase in the living body are known.

Recently,  $\gamma$ -glutamyltranspeptidase is receiving attention as a new tumor marker, particularly for hepatic cancer, because of the fact that the blood of patients with hepatic cancer contains glycoproteins carrying different sugar-chain structure compared with normal subjects. However, the results with this  $\gamma$ -glutamyltranspeptidase are not better than with AFP, CEA and others as tumor markers.

No conclusions can be drawn from these two above mentioned literature reports with respect to the diagnosis of hepatic cancer, i.e. a problem to which the object of the present invention is directed.

According to the invention this object is solved by the method as claimed, i.e. a method for diagnosing hepatocirrhosis or hepatic cancer which comprises the following steps:

- (a) adding fluorescence-labelled GnGn sugar chain and UDP-GlcNAc to a serum sample to react with UDP-N-acetylglucosamine : glycoprotein N-acetylglucosaminyltransferase III (Gn-T-III) in said serum sample to produce the following compound:



(b) subjecting the resulting reaction solution containing said compound to high-performance liquid chromatography; and

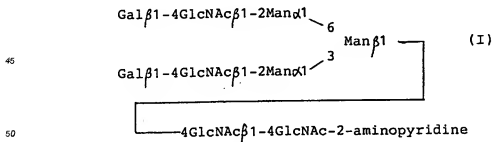
(c) examining the increase in degree of Gn-T-III activity.

Detailed studies on the change in sugar-chain structure in patients with hepatic cancer revealed that N-acetylglucosamine is attached, through  $\beta$ -1,4-linkage, to the mannose (of  $\beta$ -1,4-linkage) bound to the trimannosyl core of sugar chain of asparagine linked type. The continued investigations led to the assumption that this change might be accompanied by the increase in the amount of Gn-T-III, i.e. an enzyme capable of transferring this N-acetylglucosamine. As a result, it was demonstrated that the sera of patients suffering from hepatocirrhosis or hepatic cancer show a significantly higher Gn-T-III activity compared with normal subjects. A simple method for measuring the amount of this enzyme is realized by the present invention.

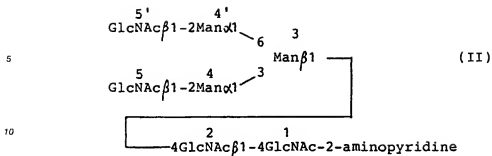
It was found by the present invention that the sera of normal subjects generally show a Gn-T-III activity as low as about  $2.0 \pm 0.5$  nmol/ml/h, while the sera of patients with hepatic cancer about 2 to 3 times the activity, the sera of patients with hepatocirrhosis about 1.5 times and the sera of patients with chronic hepatitis 1.2 times.

On page 634 of Preliminary Notes for the 60th Meeting of Japanese Biochemical Society, is described a method of measuring Gn-T-III activity, in which N-acetylglucosamine is transferred to GnGn sugar chain and the product thus formed is measured by high-performance liquid chromatography. However, it is not known at all to apply this method to the diagnosis of cancerous diseases.

In the method of this invention, the amount of Gn-T-III is preferably measured by allowing it to act upon uridine diphospho N-acetylglucosamine ( hereinafter abbreviated as UDP-GlcNAc ) and to transfer N-acetylglucosamine to GnGn sugar chain. Thus the product formed is detected by high-performance liquid chromatography. In this case, if the GnGn sugar chain is previously fluorescence-labelled, the product can be easily detected by monitoring the fluorescence intensity. The GnGn sugar chain used in this invention is isolated from human transferrin, and then pyridylaminated ( fluorescence labelling ) by the method of Hase et al. ( S. Hase et al, Journal of Biochemistry, 197-203 (1984) ), as shown by formula (I).

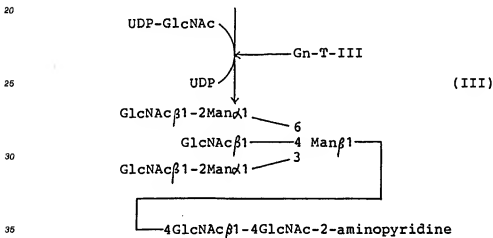


$\beta$ -Galactosidase is then allowed to act upon this sugar chain, giving pyridylaminated GnGn sugar chain of formula (II).



15 The GnGn sugar chain herein means the part of compound (II) from which 2-aminopyridine ( fluorescent substance ) is removed, and it also includes a derivative thereof in which fucose is attached to the 1-position ( GlcNAc ).

The reaction of Gn-T-III in the method of this invention is shown by the following equation (III):  
Fluorescence-labelled GnGn Sugar Chain



40 The reaction mixture was subjected to high-performance liquid chromatography, and the amount of reaction product was determined from the fluorescence-intensity, thus measuring the enzyme activity of Gn-T-III.

The amount of Gn-T-III may also be measured by other methods, such as by the antigen-antibody reaction.

#### Effects Achieved by the Invention

45 It was demonstrated that hepatic disease increases the Gn-T-III activity in the serum, and that this enzyme activity can be easily measured by allowing it to act upon UDP-GlcNAc to transfer N-acetylglucosamine to GnGn sugar chain and determining the amount of reaction product by high-performance liquid chromatography. This invention provides a simple method for diagnosing hepatic cancer based on these findings.

Presented below is an Example of this invention.

#### Example

#### 55 Reagent

250mM MES ( 2-(N-morpholino)ethanesulfonic acid monohydrate ) ( pH: 6.25 )  
400mM GlcNAc ( N-Acetylglucosamine )

20mM MnCl<sub>2</sub>  
40mM UDP-GlcNAc  
1.0% Triton X-100®

150  $\mu$ M GnGn sugar chain ( fluorescence-labelled )

Into fifty containers each containing 50  $\mu$ l of the above reagent, were added 50  $\mu$ l of sera taken from patients with primary hepatic cancer, patients with hepatocirrhosis, patients with chronic hepatitis, patients with fatty liver and normal persons ( 10 cases each ), the mixtures were incubated at 37°C for one hour, and the reaction was terminated by adding 20  $\mu$ l each of a solution containing 0.2M EDTA and 0.1M sodium borate.

Each of the reaction mixtures ( 1  $\mu$ l ) was subjected to high-performance liquid chromatography, fluorescence-intensity chromatograms were prepared, and the Gn-T-III relative activity was determined for each case.

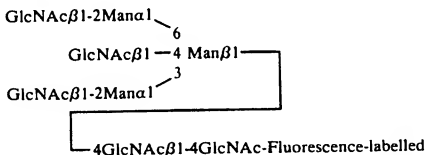
The result is shown in Table 1 below.

Table 1

	Gn-T-III Relative Activity
Serum of patients with primary hepatic cancer	3.7±2.3
Serum of patients with hepatocirrhosis	3.3±1.8
Serum of patients with chronic hepatitis	2.0±0.5
Serum of patients with fatty liver	2.0±0.5
Serum of normal persons	2.0±0.5

## Claims

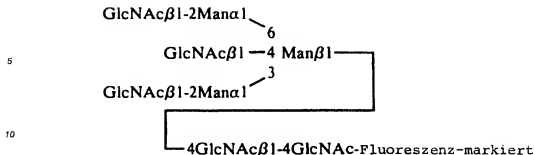
1. Method for diagnosing hepatocirrhosis or hepatic cancer which comprises the following steps:  
(a) adding fluorescence-labelled GnGn sugar chain and UDP-GlcNAc to a serum sample to react with UDP-N-acetylglucosamine:glycoprotein N-acetylglucosaminyltransferase III (Gn-T-III) in said serum sample to produce the following compound:



- (b) subjecting the resulting reaction solution containing said compound to high-performance liquid chromatography; and
- (c) examining the increase in degree of Gn-T-III activity.

## Patentansprüche

1. Verfahren zum Diagnostizieren von Leberzirrhose oder Leberkrebs, gekennzeichnet durch die folgenden Stufen:
  - (a) eine Fluoreszenz-markierte GnGn-Zuckerkette und UDP-GlcNAc werden zu einer Serumprobe gegeben zur Reaktion mit UDP-N-Acetylglucosamin : Glycoprotein-N-acetylglucosaminyltransferase III (Gn-T-III) in der Serumprobe zur Erzeugung der folgenden Verbindung:



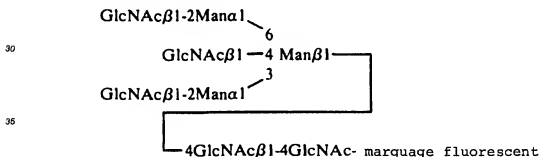
(b) die resultierende Reaktionslösung, worin die Verbindung enthalten ist, wird einer Hochleistungsflüssigchromatographie unterworfen; und

(c) die Zunahme im Grad der Gn-T-III-Aktivität wird geprüft.

#### Revendications

- 20 1. Procédé pour diagnostiquer la cirrhose du foie ou le cancer du foie, qui comprend les étapes consistant :

(a) à ajouter une chaîne de sucres GnGn marquée par un masqueur fluorescent et de l'UDP-GlcNAc à un échantillon de sérum, pour réaction avec l'UDP-N-acétylglucosamine : glycoprotéine N-acétylglucosaminyl-transférase III (Gn-T-III) se trouvant dans l'échantillon de sérum pour produire le composé suivant :



(b) à soumettre la solution réactionnelle obtenue contenant le composé à une chromatographie en phase liquide hautes performances ; et

(c) à examiner l'augmentation du degré d'activité de Gn-T-III.